

Ca 23 S AFNTQAAP 31
Cs 1 G 1

Tr2#		10	20	30	40	
Bp	1	RTITNNEMGN	HSGYDYELWK	DYGNT-SMTL	NNGGAFSAGW	N--NIGNA 45
Ca	32	KTITSNEIGV	NGGYDYELWK	DYGNT-SMTL	KNGGAFSCQW	S--NIGNA 76
Fs	1	NSSVTGNVG	SSPYHYEIWY	QGG-NNSMTF	YDNGTYKASW	N--GTNDF 44
Cs	2	RIIYDNETGT	HGGYDYELWK	DYGNT-IMEL	NDGGTFSCQW	S--NIGNA 46
Rf	1	SAADQQTRGN	VGGYDYEMWN	QNGGQASMN	PGAGSFTCSW	S--NIENF 46
Tr2	1	QTIQPGTGY	NNGYFYSYWN	DGHGGVTTYN	GPGGQFSVNW	S--NSGNF 45
Tv	1	QTIQPGTGF	NNGYFYSYWN	DGHGGVTTYN	GPGGQFSVNW	S--NSGNF 45
Th	1	QTIGPGTGY	SNGYYYSYWN	DGHAGVTTYN	GCGGSFTVNW	S--NSGNF 45
Sc	1	SGTPSSTGT	DGGYYYSWWT	DGAGDATYQN	NGGGSYTLTW	SG--NNGNL 46
An	1	S	AGINYVQNYN	GNLGDFTY-D	ESAGTFSMYW	EDGVSSDF 38
Ak	1	S	AGINYVQNYN	GNLADFTY-D	ESAGTFSMYW	EDGVSSDF 38
AT	1	S	AGINYVQNYN	QNLGDFTY-D	ESAGTFSMYW	EDGVSSDF 38
Tr1	1		ASINYDQNYQ	TGG-QVSY-	PSNTGFSVNW	N--TQDDF 34
Aa	1	RSTPSSTGE	NNGYYYSFWT	DGGGDVTTYN	GNAGSYSVEW	S--NVGNF 45
Ss	1	ATTIT-NETGY	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTRW	T--NCGNF 45
SlB	1	DTVVTNQEGT	NNGYYYSFWT	DSQGTVSMNM	GSGGQYSTSW	R--NTGNF 47
SlC	1	ATTITTNOTGT	D-GMYYSFWT	DGGGSVSMTL	NGGGSYSTQW	T--NCGNF 46
Tl	1	QTPNSEGW	HDGYYYSWWS	DGGAQATYTN	LEGCTYBISW	G--DGGNL 45
Tf	1	AVTSNETGY	HDGYFYSFWT	DAPGTVSMEL	GPGGNYSTSW	R--NTGNF 45
Bc	1		ASTDYWQNWT	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF 36
Bs	1		ASTDYWQNWT	DGGGIVNAVN	GSGGNYSVNW	S--NTGNF 36

FIGURE 1

TTTTT-42806660

FOREF-42806660

Tr2#		50		60		70		80	
Bp	46	LFRK-GKKFD	ST-RTHHQLG	NISINYNASF	N-PSGNSYLC	VYGWTQSP	90		
Ca	77	LFRK-GKKFN	DT-QTYKQLG	NISVNYNCNY	Q-PYGNSYLC	VYGWTSSP	121		
FS	45	LARV-GFKYD	EK-HTYEELGP	IDAYYKWSKO	GSAGGYNYIG	LYGWTVPD	91		
Cs	47	LFRK-GRKFN	SD-KTYQELG	DIVVEYGCDY	N-PNGNSYLC	VYGWTRNF	91		
Rf	47	LARM-GKNYD	SQKKNYKAFG	NIVLTYDVEY	T-PRGNSYMC	VYGWTRNP	92		
Tr2	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNGNSYLS	VYGWSRNP	83		
Tv	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNGNSYLS	VYGWSRNP	83		
Th	46	VGGK-GWQPG	TKNKV-----	---INFS-GS	YNPNGNSYLS	LYGWSRNP	83		
Sc	47	VGGK-GWNPG	AASRS-----	---ISYS-GT	YQPNGNSYLS	VYGWTRSS	84		
An	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVVNYP	77		
Ak	39	VVGL-GWTTG	SSNA-----	---ITYSAEY	SASGSSSYLA	VYGVVNYP	77		
At	39	VVGLGGWTTG	SSNA-----	---ITYSAEY	SASGSASYLA	VYGVVNYP	77		
Tr1	35	VVGW-GWTTG	SSAP-----	---INFGGSF	SVNSGTGLLS	VYGWSTNP	72		
Aa	46	VGGK-GWNPG	SAKD-----	---ITYSGNF	T-PSGNGYLS	VYGWTTDP	83		
Ss	46	VAGK-GWANG	GR-RT-----	---VRYT-GW	FNPSGNGYGC	LYGWTSNP	82		
SlB	48	VAGK-GWANG	GR-RT-----	---VOYS-GS	FNPSGNAYLA	LYGWTSNP	84		
SlC	47	VAGK-GWSTG	DGN-----	---VRYN-GY	FNPVGNGYGC	LYGWTSNP	82		
Tl	46	VGGK-GWNPG	LNARA-----	---IHFE-GV	YQPNGNSYLA	VYGWTRNP	83		
Tf	46	VAGK-GWATG	GR-RT-----	---VTYS-AS	FNPSGNAYLT	LYGWTRNP	82		
Bc	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGWTRSP	75		
Bs	37	VVGK-GWTTG	SPFRT-----	---INYNAGV	WAPNGNGYLT	LYGWTRSP	75		

Tr2#		90		100		110		120		130
Bp	91	LA E Y V I V D S W	GTYR-PT--G	AYKGSFYADG	GT Y D I Y E T T R	VN O PS I I G	135			
Ca	122	L V E Y Y I V D S W	GSWRPP--GG	TSKGTITVDG	GI Y D I Y E T T R	IN O PS I IOG	167			
FS	92	L V E Y Y I V D D W	FNKPGANLLG	QRKGEFTVDG	DT Y E I W O N T R	VO O PS I IKG	139			
Cs	92	L V E Y Y I V E S W	GSWRPP--GA	TPKGTITQWMA	GT Y E I Y E T T R	VN O PS I IDG	138			
Rf	93	L M E Y Y I V E GW	GDWRPPGNDG	EVKGTVSANG	NT Y D I R K T M R	YN O PS I LDG	140			
Tr2	84	L I E Y Y I V E NF	GTYN-PSTGA	TKLGEVTS D G	SV Y D I Y R T O R	VN O PS I I G	130			
Tv	84	L I E Y Y I V E NF	GTYN-PSTGA	TKLGEVTS D G	SV Y D I Y R T O R	VN O PS I I G	130			
Th	84	L I E Y Y I V E NF	GTYN-PSTGA	TKLGEVTS D G	SV Y D I Y R T O R	VN O PS I I G	130			
Sc	85	L I E Y Y I V E SY	GSYD-PSSAA	SHKGSVTCNG	AT Y D I L S T W R	YN A PS I IDG	131			
An	78	QA E Y Y I V E D Y	GDYN-PCSSA	TSLGTVYS D G	ST Y Q V CT D T R	IN E PS I ITG	124			
Ak	78	QA E Y Y I V E D Y	GDYN-PCSSA	TSLGTVYS D G	ST Y Q V CT D T R	TN E PS I ITG	124			
At	78	QA E Y Y I V E D Y	GDYN-PCSSA	TSLGTVYS D G	ST Y Q V CT D T R	IN E PS I ITG	124			
Tr1	73	L V E Y Y I M E D N	HNY--PAQ-G	TVKGTVTSDG	AT Y T I W E N T R	VN E PS I IOG	117			
Aa	84	L I E Y Y I V E SY	GDYN-PGSGG	TTRGNVSS D G	SV Y D I Y T A T R	TN A PS I IDG	130			
Ss	83	L V E Y Y I V D NW	GSYR-PT--G	ETRGTVHSDG	GT Y D I Y K T T R	YN A PS V EA	127			
SlB	85	L V E Y Y I V D NW	GTYR-PT--G	EYKGTVTSDG	GT Y D I Y K T T R	VN K PS V EG	129			
SlC	83	L V E Y Y I V D NW	GSYR-PT--G	TYKGTVSS D G	GT Y D I Y Q T T R	YN A PS V EG	127			
Tl	84	L V E Y Y I V E NF	GTYD-PSSGA	TDLGTV E C D G	SI Y RLG K T T R	VN A PS I IDG	130			
Tf	83	L V E Y Y I V E SW	GTYR-PT--G	TYMGTVT T D G	GT Y D I Y K T T R	YN A PS I EG	127			
Bc	76	L I E Y Y V V D SW	GTYR-PT--G	TYKGTVKSDG	GT Y D I Y T T T R	YN A PS I IDG	120			
Bs	76	L I E Y Y V V D SW	GTYR-PT--G	TYKGTVKSDG	GT Y D I Y T T T R	YN A PS I IDG	120			

FIGURE 1 CONT'D

FOI 21-01-480660

Tr2#	140	150	160
Bp 136	-IATEKQYWS VRQTKRTS--	-----GTVS	VSAHPRKWES LGMPM-GK 174
Ca 168	-NTTEKQYWS VRRTKRTS--	-----GTIS	VSKHFAAWES KGMPM-GK 206
Fs 140	-TQTFQYFVS VRKSARSC--	-----GHID	ITAHMKKWE LGMKM-GK 178
Cs 139	-TATEQQYWS VRTSKRTS--	-----GTIS	VTEHFKOWER MGMRM-GK 177
Rf 141	-TATEQQYWS VRQTSQSANN	QTNMKGTD	VSKHFDASAS AGLDMSGT 187
Tr2 131	-TATEQQYWS VERNHR-S-S	-----GSVN	TANHFNAWAQ QGLTL-GT 168
Tv 131	-TATEQQYWS VERTHR-S-S	-----GSVN	TANHFNAWAQ QGLTL-GT 168
Th 131	-TATEQQYWS VERNHR-S-S	-----GSVN	TANHFNAWAS HGLTL-GT 168
Sc 132	-TQTFQYFVS VRNPKKAPGG	SIS---GTV	VOCHFDANKG LGMNLGSE 175
An 125	-TSTFTQYFS VRESTRTS--	-----GTVT	VANHFNFWAQ HGFEN-SD 163
Ak 125	-TSTFTQYFS VRESTRTS--	-----GTVT	VANHFNFWAQ HGFEN-SD 163
At 125	-TSTFTQYFS VRESTRTS--	-----GTVT	VANHFNFWAH HGFEN-SD 163
Tr1 118	-TATEQQYIS VRNSPR-T-S	-----GTVT	VQNHFN-WAS LGLHLGOM 155
Aa 131	-TQTFQYFVS VRQNKV-VG-	-----GTVT	TSNHFNWAH LGMNL-GT 168
Ss 128	-PAAFDQYWS VRQSKVT--S	-----GTIT	TGNHFDARAR AGMNMGNF 168
SLB 130	TR-TEDQYWS VRQSKR-TG-	-----GTIT	TGNHFDARAR AGMPLGNF 168
SLC 128	TK-TEDQYWS VRQSKVTSGS	-----GTIT	TGNHFDARAR AGMNMGNF 168
Tl 131	TQ-TEDQYWS VRQDKR-T-S	-----GTVQ	TGCHFDARAR AGLNVNGD 169
Tf 128	TR-TEDQYWS VRQSKRTS--	-----GTIT	AGNHFDARAR HGMHLGTH 166
Bc 121	DRTTFTQYWS VRQSKRPTGS	N-----ATIT	FTNHVNNAWS HGMNLGSN 163
Bs 121	DRTTFTQYWS VRQSKRPTGS	N-----ATIT	FSNHVNNAWS HGMNLGSN 163

Tr2#	170	180	190
Bp 175	MYETAFTVEG YQSSGSANVM	TNQLFIGN	201
Ca 207	MHETAFNIEG YQSSGKADVN	SMSINIGK	233
Fs 179	MYEAKVLVEA GGGSGSFDV-	TYFKMT	203
Cs 178	MYEVALTVEG YQSSGYANVY	KNEIRIGANP....	
Rf 188	LYEVSLNIEG YRSNGSANVK	SVSV	211
Tr2 169	MDYQIVAVEG YFSSGSASI-	TVS	190
Tv 169	MDYQIVAVEG YFSSGSASI-	TVS	190
Th 169	MDYQIVAVEG YFSSGSASI-	TVS	190
Sc 176	HNYQIVATEG YQSSGTATI-	TVT	197
An 164	FNYQVMAVEA WSGAGSASV-	TISS	185
Ak 164	FNYQVMAVEA WSGAGSASV-	TISS	185
At 164	FNYQVMAVEA WSGAGSAAV-	TISS	185
Tr1 157	MNYQVVAVEG WGGSGSASQ-	SVSN	178
Aa 169	HNYQILATEG YQSSGSSSI-	TIQ	190
Ss 167	RYYMINATEG YQSSGSSSI-	TVSG	189
SLB 169	SYMINATEG YQSSGTSSI-	NVGG.....	
SLC 169	RYYMINATEG YQSSGSSNI-	TVSG	191
Tl 170	HYYQIVATEG YFSSGYARI-	TVADVG	194
Tf 167	D-YMIMATEG YQSSGSSNVT	LGTS.....	
Bc 164	WAYQVMATEG YQSSGSSNV-	TVW	185
Bs 164	WAYQVMATEG YQSSGSSNV-	TVW	185

FIGURE 1 CONT'D

Bp *Bacillus pumilus*
Ca *Clostridium acetobutylicum* P262 XynB
Cs *Clostridium stercorarium* xynA
Rf *Ruminococcus flavefaciens*
Tr2 *Trichoderma reesei* XYN II
Tv *Trichoderma viride*
Th *Trichoderma harzianum*
Sc *Schizophyllum commune* Xylanase A
An *Aspergillus niger*, var. *awamori*
Ak *Aspergillus kawachii* XynC
At *Aspergillus tubigensis*
Tr1 *Trichoderma reesei* XYN I
Aa *Aspergillus awamori* var. *kawachi* Xyn B
Fs *Fibrobacter sccinogenes* XYN II
Ss *Streptomyces* sp. 36a
SlB *Streptomyces lividans* Xln B
SlC *Streptomyces lividans* Xln C
Tl *Thermomyces lanuginosus* Xyn
Tf *Thermomonospora fusca* TfxA
Bc *Bacillus circulans*
Bs *Bacillus subtilis*

FIGURE 1 CONT'D

FOI274-4280660

5' -CT AGC TAA GGA GG CTG CAG ATG
 G ATT CCT CC GAC GTC TAC
 NheI | PstI

TrX-1
 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16
 Q T I Q P G T G Y N N G Y F Y S
 CAA ACA ATA CAA CCA GGA ACC GGT TAC AAC AAC GGT TAC TTT TAC AGC
 GTT TGT TAT GTT GGT CCT TGG CCA ATG TTG TTG CCA ATG AAA ATG TCG
 TrX-8 AgeI

XyTv-2
 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
 Y W N D G H G G V T Y T N G P G
 TAT TGG AAC GAT GGC CAT GGT GGT GTT ACC TAT ACA AAC GGG CCC GGA
 ATA ACC TTG CTA CCG GTA CCA CAA TGG ATA TGT TTG CCC GGG CCT
 NcoI XyTv-7 ApaI

33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48
 G Q F S V N W S N S G N F V G G
 GGC CAA TTT AGC GTC AAT TGG TCT AAC TCC GGA AAC TTC GTA GGT GGA
 CCG GTT AAA TCG CAG TTA ACC AGA TTG AGG CCT TTG AAG CAT CCA CCT
 MunI BspEI

TrX-3
 49 50 51 52 53 54 55 56 57 58 59 60 61 62 63 64
 K G W Q P G T K N K V I N F S G
 AAA GGT TGG CAA CCC GGG ACC AAA AAT AAG GTG ATC AAC TTC TCT GGA
 TTT CCA ACC GTT GGG CCC TGG TTT TTA TTC CAC TAG TTG AAG AGA CCT
 XmaI TrX-6

65 66 67 68 69 70 71 72 73 74 75 76 77 78 79 80
 S Y N P N G N S Y L S V Y G W S
 TCT TAT AAT CCG AAT GGG AAT TCA TAC TTA AGC GTC TAT GGC TGG TCT
 AGA ATA TTA GGC TTA CCC TTA AGT ATG AAT TCG CAG ATA CCG ACC AGA
 EcoRI AflII

XyTv-4
 81 82 83 84 85 86 87 88 89 90 91 92 93 94 95
 R N P L I E Y Y I V E N F G T
 AGA AAC CCA CTG AAT GAA TAT TAC ATT GTC GAA AAT TTC GGT AC
 TCT TTG GGT GAC TAA CTT ATA ATG TAA CAG CTT TTA AAG C
 Xba I XyTv-5 KpnI

FIGURE 2

TOTAL = 42806660

XyTv-101															
V	D	92	93	94	95	96	97	98	99	100	101	102	103	104	105
TC	GAC	AAT	TTC	GGT	ACC	TAC	AAT	CCG	AGT	ACC	GGC	GCC	ACA	AAA	TTA
3'-G	TTA	AAG	CCA	TGG	ATG	TTA	GGC	TCA	TGG	CCG	CCG	TGT	TTT	AAT	
SallI		KpnI				XyTv-110				KasI/NarI					
XyTv-102															
106	107	108	109	110	111	112	113	114	115	116	117	118	119	120	121
G	E	V	T	S	D	G	S	V	Y	D	I	Y	R	T	Q
GGC	GAA	GTC	ACT	AGT	GAT	GGA	TCC	GTA	TAT	GAT	ATC	TAC	CGT	ACC	CAA
CCG	CTT	CAG	TGA	TCA	CTA	CCT	AGG	CAT	ATA	CTA	TAG	ATG	GCA	TGG	GTT
				SpeI		BamHI				XyTv-109					
TrX-103															
122	123	124	125	126	127	128	129	130	131	132	133	134	135	136	137
R	V	N	Q	P	S	I	I	G	T	A	T	F	Y	Q	Y
CGC	GTT	AAT	CAG	CCA	TCG	ATC	ATT	GGA	ACC	GCC	ACC	TTT	TAT	CAG	TAC
CGC	CAA	TTA	GTC	GGT	AGC	TAG	TAA	CCT	TGG	CGG	TGG	AAA	ATA	GTC	ATG
MluI		ClaI													
138	139	140	141	142	143	144	145	146	147	148	149	150	151	152	153
W	S	V	R	R	N	H	R	S	S	G	S	V	N	T	A
TGG	AGT	GTT	AGA	CGT	AAT	CAT	CGG	AGC	TCC	GGT	TCG	GTT	AAT	ACT	GCG
ACC	TCA	CAA	TCT	GCA	TTA	GTA	GCC	TCG	AGG	CCA	AGC	CAA	TTA	TGA	CGC
TrX-108				SacI											
XyTv-104															
154	155	156	157	158	159	160	161	162	163	164	165	166	167	168	169
N	H	F	N	A	W	A	Q	Q	G	L	T	L	G	T	M
AAT	CAC	TTT	AAT	GCA	TGG	GCA	CAG	CAA	GGG	TTA	ACC	CTA	GGT	ACA	ATG
TTA	GTG	AAA	TTA	CGT	ACC	CGT	GTC	GTT	CCC	AAT	TGG	GAT	CCA	TGT	TAC
				NsiI				XyTv-107				AvrII			
XyTv-105															
170	171	172	173	174	175	176	177	178	179	180	181	182	183	184	185
D	Y	Q	I	V	A	V	E	G	Y	F	S	S	G	S	A
GAT	TAT	CAA	ATC	GTA	GCG	GTG	GAA	GGC	TAC	TTC	TCG	AGT	GGT	TCC	GCT
CTA	ATA	GTT	TAG	CAT	CGC	CAC	CTT	CCG	ATG	AAG	AGC	TCA	CCA	AGG	CGA
				XyTv-106				XhoI							
186	187	188	189	190											
S	I	T	V	S											
AGT	ATT	ACA	GTG	AGC	TAA	A									
TCA	TAA	TGT	CAC	TCG	ATT	TCT	AG-5'								
				BglII											

FIGURE 2 CONT'D

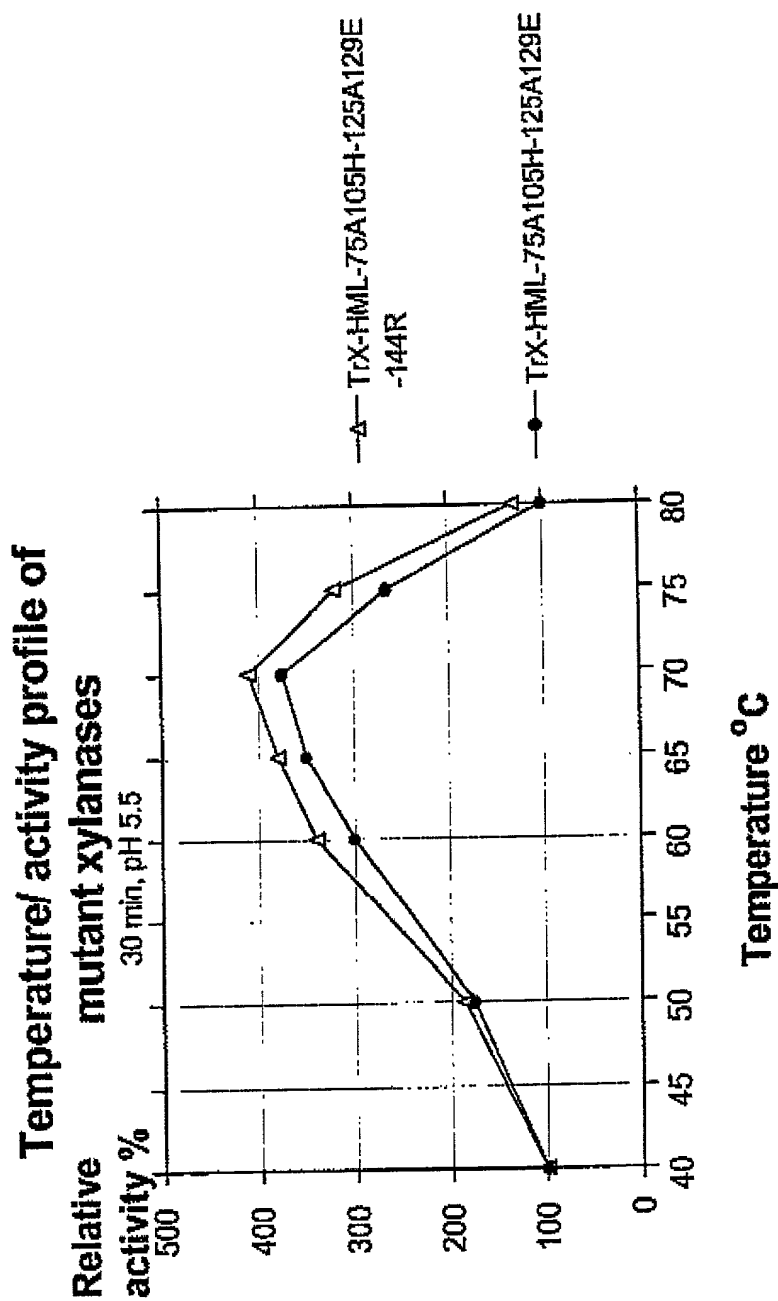


Figure 3

TOT2T*4806660

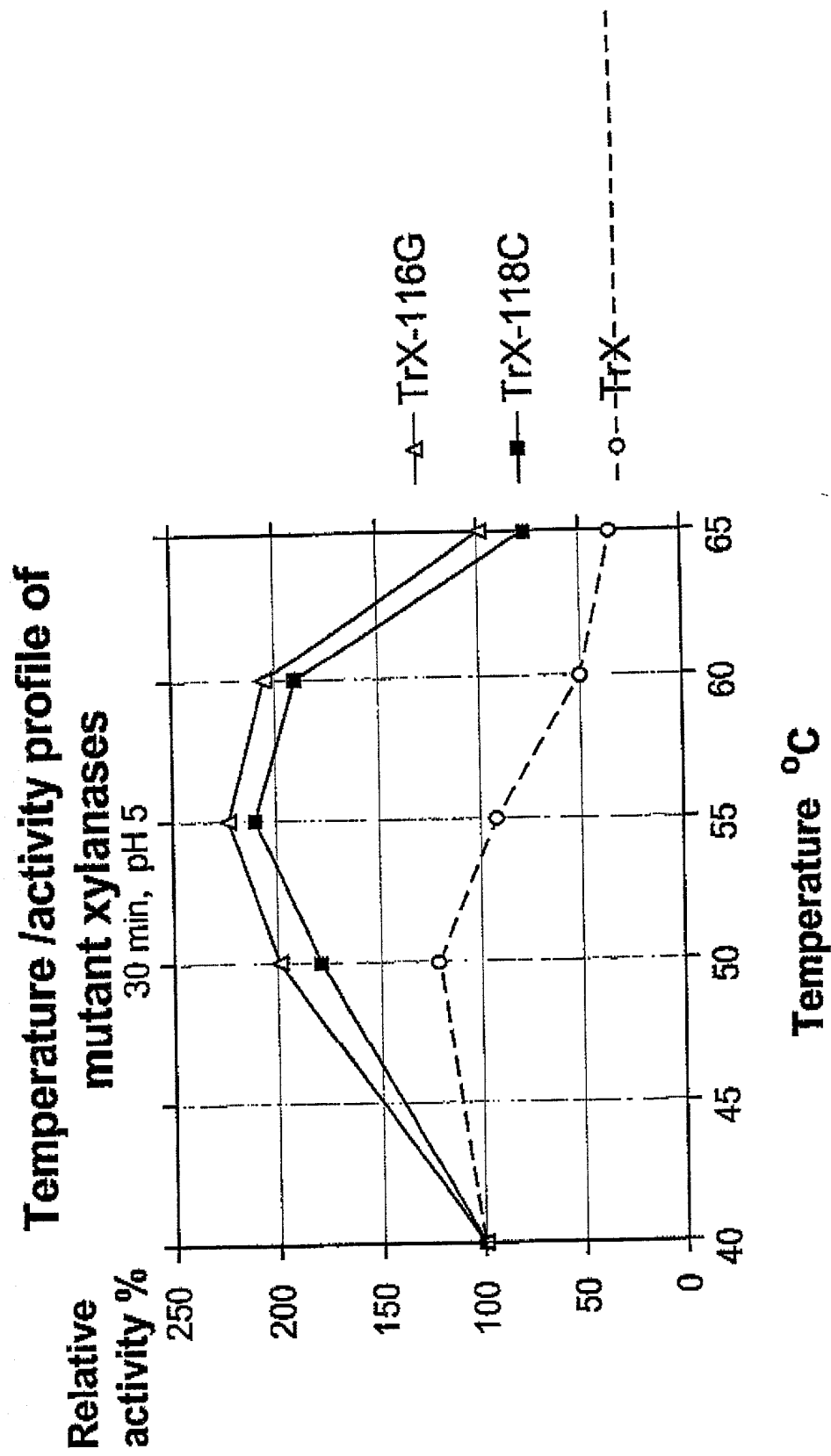


Figure 4

TOFTT-42806660

Effect of Temperature on the activity of mutant xylanase

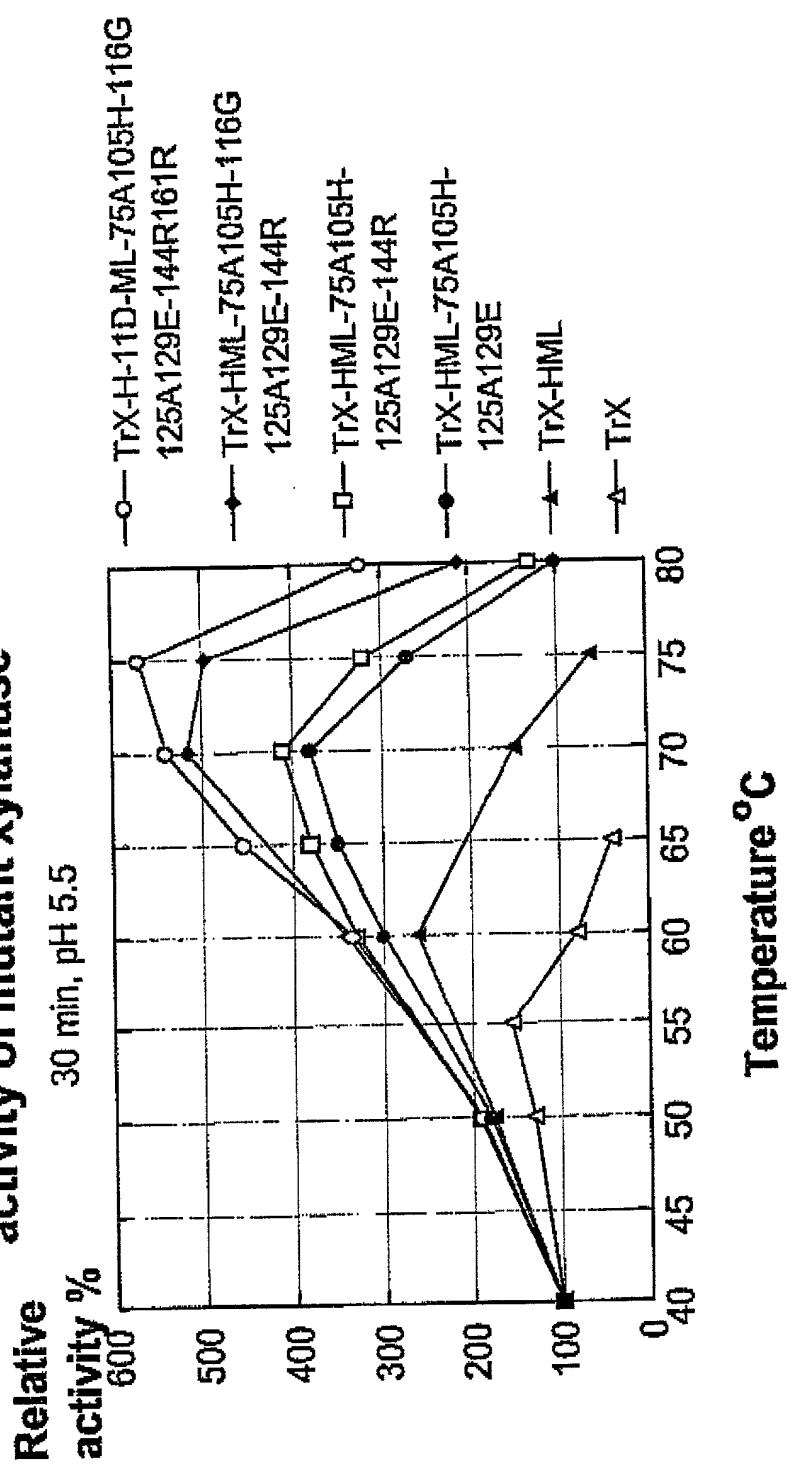


Figure 5

TOT2TT 42306660

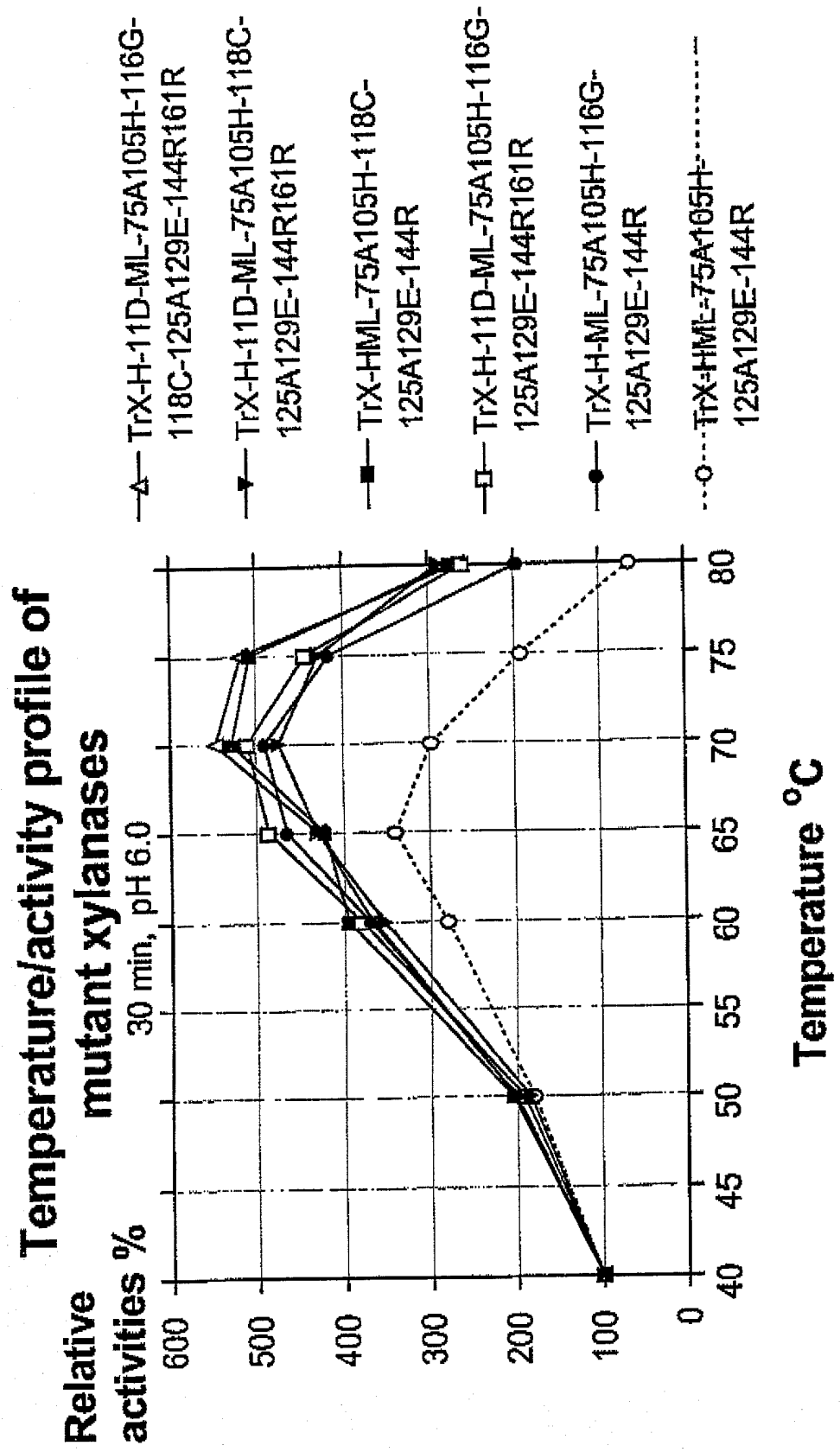


Figure 6

Temperature/activity profile of mutant xylanases

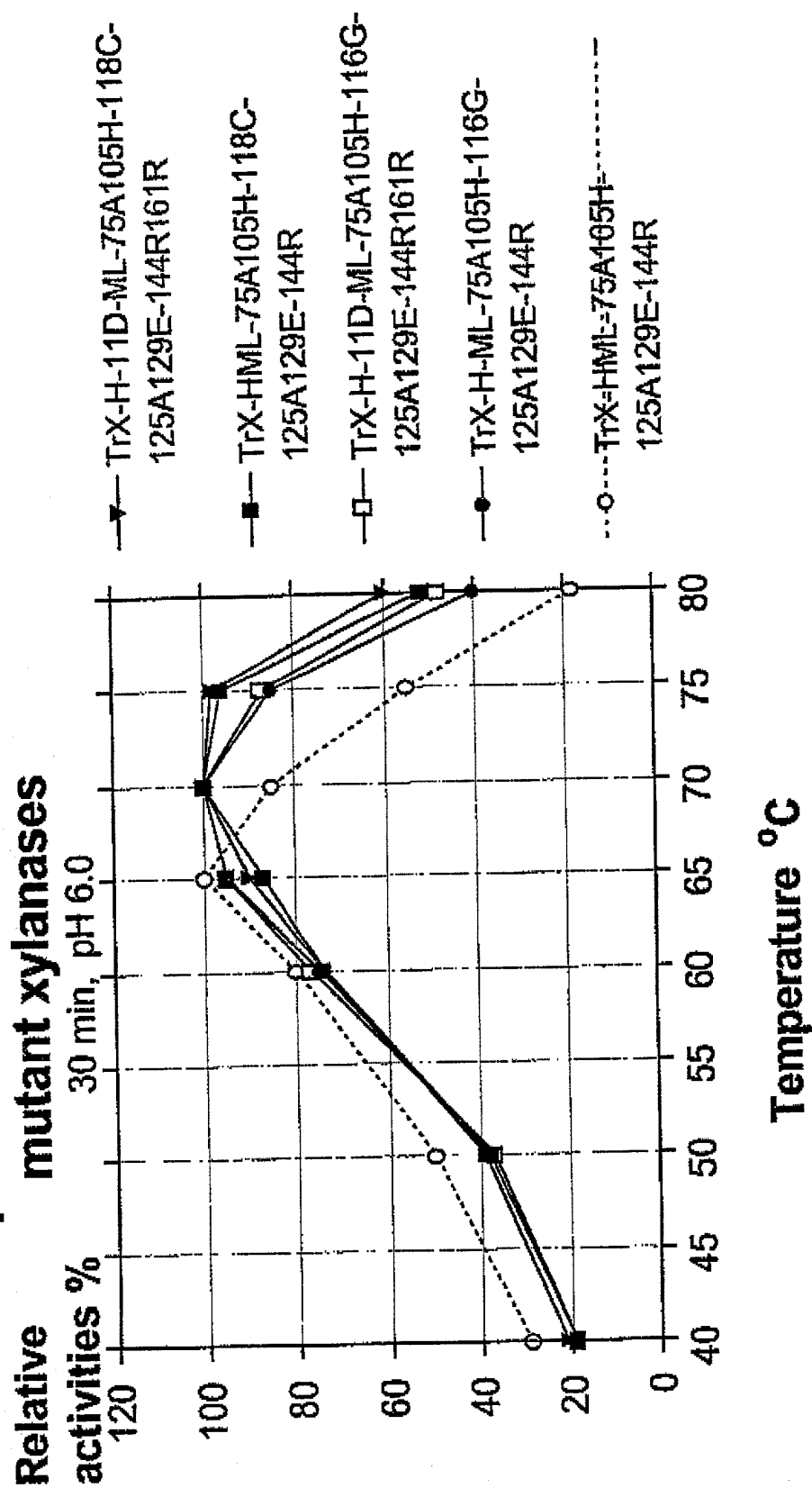


Figure 7

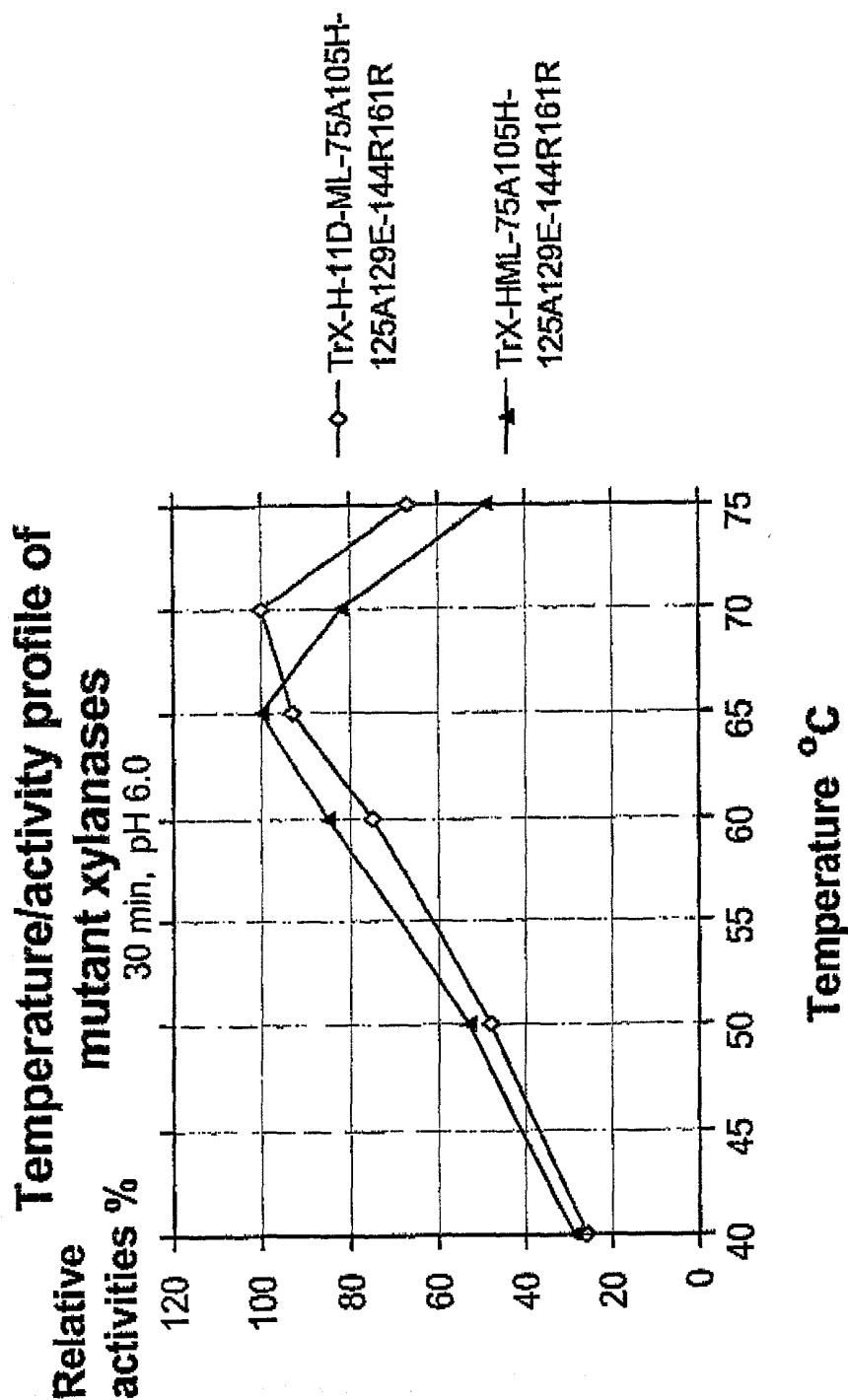


Figure 8

FOI217-1430660

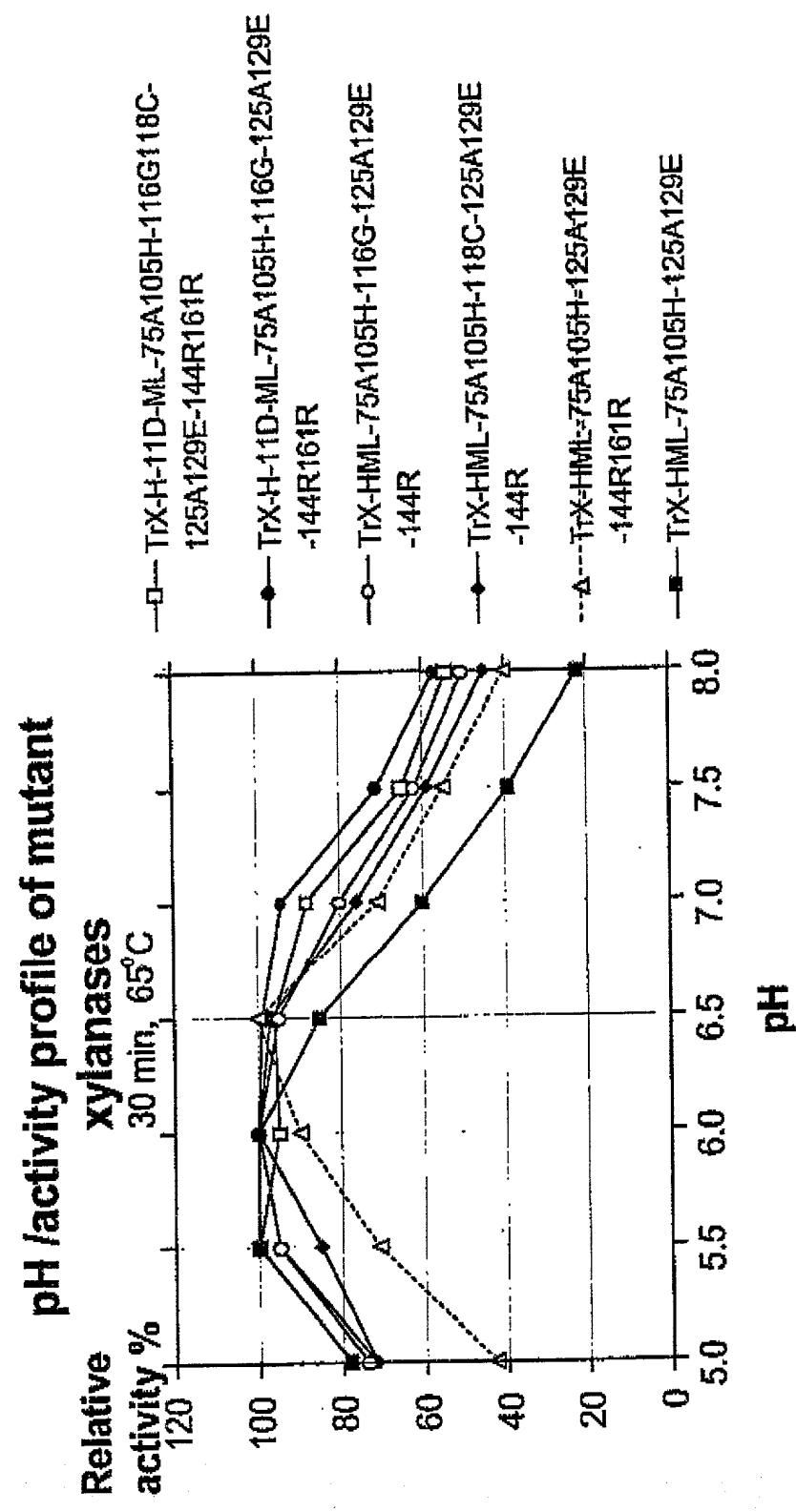


Figure 9

pH/activity profile of mutant

Relative
activity %

xylanases
30 min, 50°C

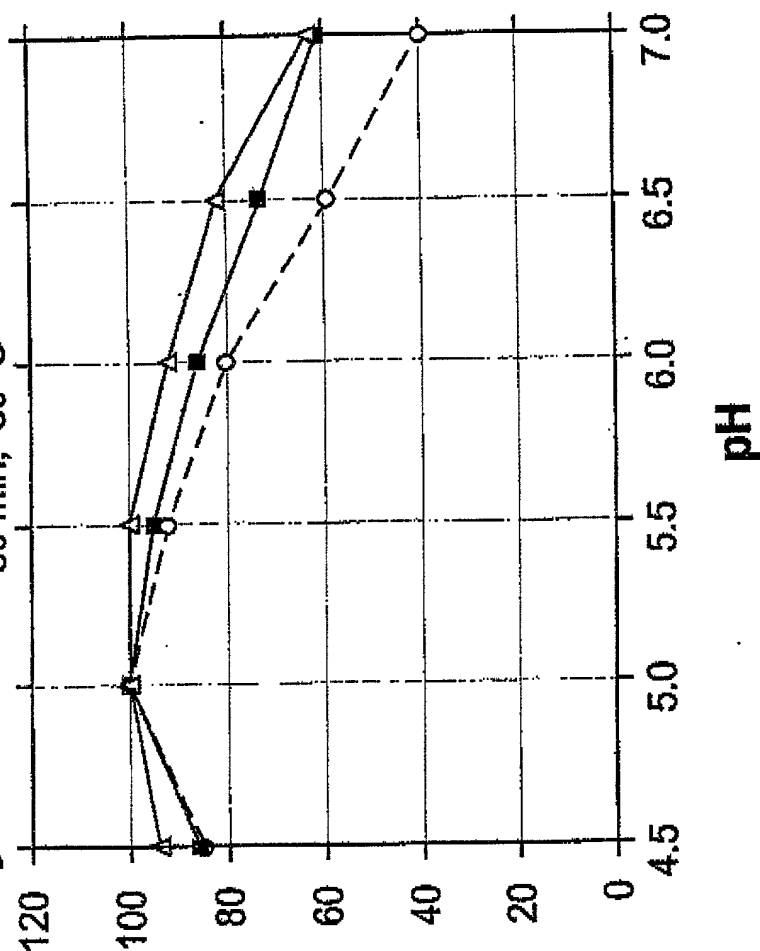


Figure 10

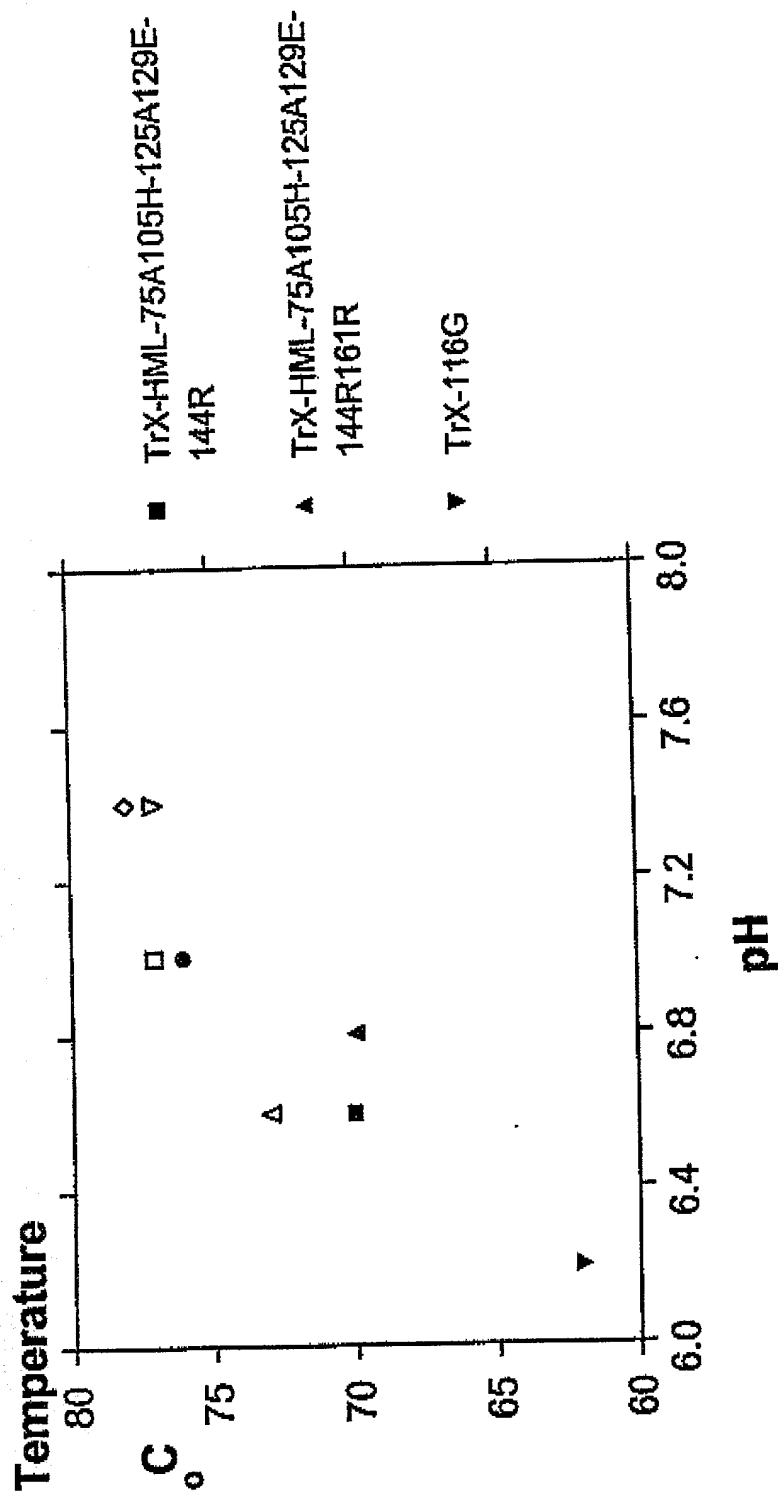


Figure 11

- TrX-HML-75A105H-116G-125A129E-144R
- TrX-HML-75A105H-118C-125A129E-144R
- △ TrX-H-11D-ML-75A105H-125A129E-144R161R
- ▽ TrX-H-11D-ML-75A105H-116G-125A129E-144R161R
- ◇ TrX-H-11D-ML-75A105H-118C-125A129E-144R161R